

THE EFFECT OF EXERCISE INTENSITY ON SERUM LEPTIN AND C-REACTIVE PROTEIN LEVELS

Te-Hung Tsao¹, Chin-Hsing Hsu¹, Chang-Bin Yang², Tzi-Li Liou¹

¹Department of Recreation Sports and Health Promotion, National Pingtung University of Science and Technology, Pingtung, TAIWAN

²Physical Education Section, National Ocean University, Keelung, TAIWAN

Recently, serum leptin and C-reactive protein (CRP) levels have been regarded as independent predictive factors for heart disease. Although exercise intensity and duration may influence leptin and CRP concentrations, few studies have investigated this. In addition, leptin and CRP exhibit trends (downward and upward, respectively) after an acute bout of aerobic exercise. There seems to be a negative association between them, which may differ from the baseline; however, no study has tested this assumption. Therefore, we investigated the effect of exercise intensity on serum leptin and CRP levels and compared changes and differences in both relationships with different exercise intensities. In addition to the $\dot{V}O_{2max}$ test, 13 male subjects (21.5 ± 1.8 years old, $18.5 \pm 4.0\%$ body fat, $55.0 \pm 3.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ $\dot{V}O_{2max}$) exercised at two other exercise intensities ($85\% \dot{V}O_{2max}$ and $65\% \dot{V}O_{2max}$) in a randomized order. Blood samples were collected before and immediately after each trial to analyze pre- and post-exercise leptin and CRP concentrations in the three trials. While there were no significant differences in post-exercise leptin and CRP levels among the different exercise intensities, there were significant differences between leptin and CRP concentrations before and after exercise bouts corresponding to 65% and $85\% \dot{V}O_{2max}$. In addition, post-exercise leptin and CRP levels were not associated. The results of this study suggest that leptin and CRP do not differ among different exercise intensities. Alteration in CRP and body fat percentage did not contribute to the change in leptin in these acute exercise models. [*J Exerc Sci Fit* • Vol 7 • No 2 • 98–103 • 2009]

Keywords: exercise time, heart disease, percent body fat, $\dot{V}O_{2max}$ test

Introduction

Leptin, an obese (Ob) gene protein mainly secreted from adipose tissue, has been related to an increase in energy consumption, thermogenesis, and suppression of appetite (Campfield et al. 1995; Halaas et al. 1995; Zhang et al. 1994). In addition, leptin concentration is significantly associated with body mass index (BMI)

and percent body fat (Boden et al. 1996; Hickey et al. 1996). After strenuous exercise, leptin levels are significantly lower than at baseline (Leal-Cerro et al. 1998; Landt et al. 1997). However, the mechanisms explaining or factors relating to leptin during exercise have not been elucidated. Consequently, studies on the effect of exercise on leptin are of interest. However, it is hard to control or maintain consistent exercise intensity in actual competition because athletes change their exercise intensity for many reasons during competition. As a result, it remains unclear if exercise intensity is a potential factor affecting leptin during exercise (Desgorces et al. 2004).

Although there are few studies on the effect of exercise intensity on leptin levels, some factors associated with leptin have been investigated at rest, such



ELSEVIER

Corresponding Author

Te-Hung Tsao, Department of Recreation Sports and Health Promotion, National Pingtung University of Science and Technology, 1 Hseuh-Fu Road, Nei-Pu Township, Pingtung 912, TAIWAN.

Tel: (+886) 8-7703202 ext. 6484

Fax: (+886) 8-7740536

E-mail: thtsao@mail.npust.edu.tw

as C-reactive protein (CRP) (Chen et al. 2006). CRP is an acute-phase reactant and is regarded as an independent factor for cardiac events (Danesh et al. 1999; Tracy et al. 1997). Furthermore, studies concerning the effect of exercise on CRP concentrations have indicated that CRP concentrations rise after strenuous exercise when compared to the resting values (Siegel et al. 2001; Taylor et al. 1987). Recently, leptin has also been regarded as an independent factor for cardiovascular disease (Sader et al. 2003; Söderberg et al. 1999; Leyva et al. 1998). According to the literature, both leptin and CRP have similar functions as predictive factors for heart disease.

Although leptin and CRP play similar roles in predicting heart disease, there is little information on the relationship between them or their effects on each other (Ble et al. 2005; Shamsuzzaman et al. 2004). In addition, these studies observed that leptin levels decrease and CRP levels increase as a result of acute exercise when compared to baseline levels. There would appear to be a negative relationship between leptin and CRP, but it is not known if this is moderated by exercise intensity. Although Desgorces et al. (2004) surmised the potential effect of exercise intensity, few studies have discussed the effect of different exercise intensities on leptin and CRP levels. In addition, while several papers have reported that an association exists between leptin and CRP at baseline, little information is available on the relationship or interactions between leptin and CRP before and after exercise.

The purpose of this study was therefore to examine the effects of different exercise intensities on leptin and CRP levels. It was hypothesized that the different exercise intensities would elicit significant differences between these two variables. In addition, we wished to explore the relationships between leptin and CRP levels at rest and after exercise at moderate to high exercise intensities.

Methods

Exercise protocol

Approval for this study was obtained from the Ethics Committee of Pingtung Christian Hospital, and the same hospital was in charge of a health check of all subjects in order to ensure that each participant was free of metabolic and cardiovascular diseases. Thirteen physically active males participated in this study. They were neither drinkers nor smokers. After reading and signing an informed consent form, the maximal oxygen

consumption ($\dot{V}O_{2max}$) of all subjects was measured by Bruce's treadmill protocol (American College of Sports Medicine 2000) using a Vmax29 from SensorMedics Corporation (Yorba Linda, CA, USA). The following criteria were met to ensure that subjects achieved maximal oxygen uptake: (1) respiratory exchange ratio > 1.1 with a $\dot{V}O_2$ plateau despite an increase in exercise intensity (speed or grade); (2) heart rate within 10 beats \cdot min⁻¹ of the age-predicted maximum (Vantage XL Polar System; Polar Electro Inc., Port Washington, NY, USA); and (3) rating of perceived exertion > 18 on the Borg Scale (Borg 1998). With regard to body fat, the body density of all subjects was estimated by skinfold thickness (Harpندن, West Sussex, UK) gauged at four sites—biceps, triceps, subscapular, and suprailiac (Durnin & Womersley 1974)—by the same experienced person and the body density was converted to %body fat using Siri's (1961) equation.

Participants completed two different bouts of exercise at exercise intensities corresponding to 65% and 85% $\dot{V}O_{2max}$ for 30 minutes in a randomized order after participants did some stretches and ran at their individual comfortable speed for 3–5 minutes, separated by 7–10 days. For the duration of the two different exercise sessions, participants also wore a mask to collect expired gas to monitor the exercise intensity and maintain the prescribed % $\dot{V}O_{2max}$ within $\pm 5\%$. Evaluation of perceived exertion using the Borg 6-20 PRE scale (Borg 1998) was also performed to record participants' sensation of exercise intensity during both sessions. For 24 hours before each trial, including the $\dot{V}O_{2max}$ test, participants were not allowed to undertake any exercise. All experimental trials were performed at the same time of day to avoid circadian variation. All subjects were free of injuries or infection for at least 5–7 days prior to the $\dot{V}O_{2max}$ test and each exercise session.

Diet

Diet was recorded 1 day before and on the day of the $\dot{V}O_{2max}$ test. All food for the 65% and 85% $\dot{V}O_{2max}$ trials was provided and verified by the previous individual dietary records. The mean energy consumption of subjects for 1 day was measured using the NetWisp 3.0 (Tinuviel Software Systems, Cheshire, UK). Beverages with caffeine or alcohol were not allowed during the test sessions, but all participants had access to water. To reduce any possible confounding factors from the diet, we provided subjects with breakfast and lunch and held each exercise procedure at the same time of day. Subjects reported to the laboratory about 07:30

each day of a scheduled exercise intensity bout and were offered breakfast and later had lunch at about 11:30 according to the data of the diet record of the maximal oxygen test. After about 3 hours, blood samples of the subjects at rest were taken. Then, after warming-up, the participants proceeded to finish 30 minutes of running at each different level on a treadmill. On arrival at the laboratory, subjects were free to read newspapers, magazines, books, listen to music, watch television, or rest. When subjects finished the exercise tests, blood samples were immediately collected for analysis.

Blood sampling

Blood samples were collected before and immediately after each exercise session, including the $\dot{V}O_{2\max}$ test. In all participants, 10 mL of blood was drawn from the basilic vein before and after each trial. All samples were thawed only once before analysis. The drawn blood was separated into two tubes: one plain tube for serum and the other with EDTA for the potential changes in hemoglobin and hematocrit (Micro Biff-II; Beckman Coulter Inc., Fullerton, CA, USA), before and after each bout of exercise. The hemoconcentration had a small change from -2.3% to 1.9% , but there were no differences between before and after the three trials by *t* test analysis. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C for subsequent analysis. Leptin and CRP concentrations were determined using an enzyme-linked immunosorbent assay from Linco Research Inc., (St Charles, MO, USA) and Immulite (Diagnostics Products Corp., Los Angeles, CA, USA), respectively. All blood samples were analyzed in duplicate. The intra- and interassay coefficients of variation of leptin and CRP were all $<5\%$.

Statistical analysis

Mauchly's sphericity test was used to check for normality and homogeneity of variance before statistical analysis. Violations of the assumption of sphericity were corrected using the Huynh-Feldt adjustment. A two-way (intensity \times time) analysis of variance for repeated measures was used to compare differences in leptin and CRP levels among the three exercise intensities. Tukey's *post hoc* test was applied when a significant *F* ratio was detected. A paired-samples Student's *t* test was used to identify differences between pre- and post-exercise. In addition, Pearson's product-moment correlation coefficient was applied to detect the relationships among the variables. SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used to perform the above

statistical procedures. All values are presented as mean \pm standard deviation. For all statistical comparisons, the level of significance was set at $p < 0.05$.

Results

After statistical power analysis (using G*Power 3.0, from the Institut für Experimentelle Psychologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), a sample of 13 provided the power of the test for leptin and CRP as 0.7 and 0.8, respectively. Values for age, percent body fat, BMI, and $\dot{V}O_{2\max}$ values were 21.5 ± 1.8 years, $18.5 \pm 4.0\%$, $21.2 \pm 1.5 \text{ kg} \cdot \text{m}^{-2}$, and $55.0 \pm 3.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively. Mean energy consumption for 1 day was 2885 ± 193 kcal (carbohydrate, $53.21 \pm 2.55\%$; fat, $25.53 \pm 1.81\%$; protein, $21.26 \pm 2.01\%$). Since all subjects were provided with identical meals according to the diet record on the day of the $\dot{V}O_{2\max}$ test, there were no significant differences in daily caloric intake. In addition, there were no differences in percent body fat and BMI from pre- to post-exercise. The mean rating of perceived exertion values for the exercise bouts of 65% and 85% $\dot{V}O_{2\max}$ and at the termination of the $\dot{V}O_{2\max}$ tests were 11.9 ± 1.3 , 15.0 ± 0.8 and 18.3 ± 1.4 , respectively. At baseline, leptin was significantly related to BMI, percent body fat and CRP ($r = 0.73$, 0.75 and 0.61 , respectively, $p < 0.05$).

Leptin

There were no significant differences in leptin prior to exercise in the three different exercise sessions. Post-exercise leptin levels were significantly lower than pre-exercise levels in the 65% and 85% $\dot{V}O_{2\max}$ exercise sessions (Table 1). In addition, there were no differences among the post-exercise leptin values among the three exercise sessions.

CRP

Similarly, there were no significant differences in CRP concentrations at baseline. CRP measurements also showed no significant intensity \times time interactions. Although there were no significant differences among the post-exercise CRP levels, these values were significantly higher than the pre-exercise CRP concentrations for each of the three different exercise intensities ($p < 0.05$).

Relationship between leptin and CRP

At baseline, there was a significant relationship between leptin and CRP ($r = 0.61$, $p < 0.05$). The associations

Table 1. Pre- and post-exercise levels of leptin and C-reactive protein (CRP) for 65% and 85% $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ tests in 13 male subjects*

	65% $\dot{V}O_{2max}$		85% $\dot{V}O_{2max}$		$\dot{V}O_{2max}$	
	Pre	Post	Pre	Post	Pre	Post
Leptin (ng/mL)	0.90 ± 0.20	0.69 ± 0.24 [†]	0.91 ± 0.24	0.66 ± 0.17 [†]	0.88 ± 0.24	0.65 ± 0.28
CRP (mg/L)	0.21 ± 0.06	0.35 ± 0.16 [†]	0.21 ± 0.04	0.42 ± 0.20 [†]	0.26 ± 0.05	0.79 ± 0.18 [†]

*Data presented as mean ± standard deviation; [†] $p < 0.05$, pre- vs. post-levels.

Table 2. Associations between leptin and C-reactive protein (CRP) at baseline, and after the 65% and 85% $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ tests

Parameter	<i>r</i>	<i>p</i>
Baseline	0.71 *	< 0.05
65% $\dot{V}O_{2max}$	-0.14	0.74
85% $\dot{V}O_{2max}$	-0.26	0.53
$\dot{V}O_{2max}$	0.35	0.41

* $p < 0.05$ between leptin and CRP.

between leptin and CRP levels after each exercise session for the three exercise intensities are displayed in Table 2. Although the association between leptin and CRP showed a negative trend for the 65% and 85% $\dot{V}O_{2max}$ exercise intensities and a positive trend in the $\dot{V}O_{2max}$ test, it should be noted that these relationships were not statistically significant. In addition, there was no correlation between the changes in pre- and post-exercise leptin and CRP levels in each trial.

Discussion

Although some studies have discussed the effects of exercise on leptin and CRP, the current study is the first to indicate that post-exercise leptin and CRP levels did not display comparable levels among different exercise intensities despite respective downward (leptin) and upward (CRP) trends. In addition, leptin was not associated with CRP after exercise in physically active persons in this study.

Leptin

Previous studies (Leal-Cerro et al. 1998; Landt et al. 1997), which have examined exercise-induced changes in leptin, have not fully described or monitored exercise intensity. Although there were similar results for changes in pre- and post-exercise leptin levels with a single exercise bout in this study compared to a single exercise or physical activity bout in other studies, there were no

significant differences among the three exercise intensities. Many studies (Murakami et al. 2007; Ryan et al. 2000; Kohrt et al. 1996) have indicated that body fat and its change due to exercise have a close association with leptin. However, with no significant change in percent body fat and no significant association between post-leptin levels and the change in percent body fat after a single bout of acute aerobic exercise, changes in leptin levels from pre- to post-exercise cannot be attributed to a change in percent body fat. On the other hand, the change in leptin levels in the short term (30 minutes) at 65% and 85% $\dot{V}O_{2max}$ was different from that found in the studies of Leal-Cerro et al. (1998) and Landt et al. (1997), which had exercise times > 60 minutes. However, this result due to short-term acute aerobic exercise was in agreement with the studies of Bouhlef et al. (2008) and Rahmani-nia et al. (2008). In fact, exercise intensity and duration are two important components in training or regular workout. As a result, we conjecture that leptin may change significantly when a given degree of exercise intensity is maintained for a period of time. However, more studies are needed to verify this.

CRP

CRP is an inflammatory index in the human body and a predictor of heart disease risk at rest. With regard to the effect of exercise on CRP, the results from the current study are similar to those of previous studies (Siegel et al. 2001; Taylor et al. 1987), i.e. CRP increased significantly after each acute bout of exercise at the three different intensities. Although post-exercise CRP levels showed an upward trend with higher exercise intensity, a significant difference was not detected among the different exercise intensities. Chen et al. (2006) suggested that CRP might be related to leptin because CRP binds to the receptor of leptin. This would result in a "leptin resistance" phenomenon as CRP occupies the leptin receptors and more leptin circulates in the blood. Although the exercise model in this study differed from that of Chen et al.'s study, this study provided another

direction for exploring the relationship between leptin and CRP.

Relationship between leptin and CRP after exercise

A significant positive relationship between leptin and CRP was observed at rest. This result is in line with those of previous studies (Ble et al. 2005; Shamsuzzaman et al. 2004; Kazumi et al. 2003) of people with different characteristics. On the other hand, the trends after 65% and 85% $\dot{V}O_{2max}$ between leptin and CRP were in agreement with our assumption, although they did not reach statistical significance. This study found that a 30-minute acute bout of exercise resulted in significantly decreased levels of leptin and significantly increased levels of CRP in the 65% and 85% $\dot{V}O_{2max}$ trials. Hence, a negative relationship between leptin and CRP after exercise seems to be plausible. In the $\dot{V}O_{2max}$ test, a positive trend was opposite to our hypothesis. Perhaps other potential factors affecting these two variables during exercise should be considered in further study. The duration of the exercise bout, however, might be a direction for discussion in the present study because this factor was not controlled for. All subjects could complete the 30 minutes of both the 65% and 85% $\dot{V}O_{2max}$ trials. However, the time for the $\dot{V}O_{2max}$ test depended on each individual's capacity (mean $\dot{V}O_{2max}$ test time, 12.4 ± 1.5 minutes). It is possible that the exercise time was insufficient to cause significant changes in leptin and CRP.

One should be careful in drawing conclusions from this study because the sample size was small. But since this study is the first to discuss the associations between leptin and CRP after exercise and among different exercise intensities, it can be seen as a pilot study and provide an interesting perspective on the relationship between leptin and CRP due to an acute bout of exercise. In some studies that reported the mechanisms for the relationship between leptin and CRP at rest, leptin caused the levels of some cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α (Zarkesh-Esfahani et al. 2004; Santos-Alvarez et al. 1999), to rise and then stimulate the synthesis of CRP in hepatic cells. In obese and overweight persons, this suggests that CRP might be elevated by the stimulation of the cytokines IL-6 and TNF- α , which would also increase leptin at the same time (Kern et al. 2001). However, these mechanisms might not be at play in the exercise model used in this study. First, the exercise model used in the current study is different from that in Kern et al.'s study. Second, body fat might not have contributed to the changes in leptin and CRP

concentrations because it did not differ significantly and relate to leptin and CRP after exercise. Finally, the above cytokines were not measured in this study. As a result, further research is necessary to ascertain the relationships among leptin, CRP and cytokines from different subjects, for example, elite athletes, regular exercisers and physically inactive persons, at rest and after exercise.

In conclusion, after strenuous aerobic exercise, leptin and CRP showed significant differences when compared to resting values. However, there were no significant differences in leptin or CRP levels within the different exercise intensities. In addition, these values were not related to each other after exercise. Other cytokines and different types of subjects should be included in further studies.

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